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RESEARCH ARTICLE

Effect of wastewater sludge treatment on early growth and physiological responses of willow (*Salix* spp.) and poplar (*Populus* spp.) pot-grown plants

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Abstract

Combining biomass production from fast-growing woody species with wastewater treatment is an attractive concept from the economic, energy and environmental points of view. A pilot experiment on studying the effect of wastewater treatment on the early growth and initial physiological responses of willow and poplar plants was carried out in greenhouse conditions. The survival, height growth, net photosynthesis, stomatal conductance and transpiration of two hybrid clones of black poplar (*Populus x euroamericana* (Dode) Guinier) (I-214 and I-45/51) and white willow (*S. alba* L.) pot-grown plants treated with different doses of agrochemically characterised wastewater sludge were studied during the establishment year. The height growth and number of resprouted willow shoots were also measured during the second year after cutting, at the end of the vegetation season. While the early growth height of poplar clones was markedly affected by the clone origin and wastewater sludge treatment, the latter did not considerably influence the height of the willow plants either during the establishment and the following year, but essentially increased the number of resprouted shoots during the second vegetation season. The rate of CO_2 assimilation of willow plants was significantly influenced by the wastewater sludge treatment, with plants grown at the higher doses displaying higher photosynthetic performance. The application of wastewater sludge had no essential impact on the values of the physiological parameters of the poplar plants, but particular responses were found among the clones.

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Keywords
Biosolids, fast-growing woody species, photosynthesis, soil conditioning

Introduction

The initial efforts for diversification and seeking for alternative sources of energy to the conventional ones were induced by the oil crisis in the 70ies, when the cultivation of short-rotation willow coppice introduced in Sweden promoted the intention of replacing fossil fuels with new energy sources (Dimitriou, Aronsson, 2005). The concept of using forest/wood resources for energy purposes dates back to the ancient times and has been materialised through the production of firewood. The short-rotation coppicing (SRC) is a modern version of the idea, modified as cultivation of energy plantations from fast-growing woody species (mainly *Populus* and *Salix* spp.) and managed in short rotations (2-5 years). Recently, establishing short-rotation coppicing plantations has become an increasingly popular practice in Europe as a result of a switch of EC policies towards use of renewable energy sources (Directive 2009/93/EC). As such, the final products from biomass produced by energy plantations (biofuel, energy, heat) are CO_2 neutral, thus contributing to the reduction of greenhouse gases and their impact on climate change (Heinsoo et al., 2008).

An interesting alternative to mineral fertilizers are biosolids and other industrial and agricultural byproducts (Guidi et al., 2012). Municipal wastes, especially wastewaters, can be regarded in many cases as beneficial as they add more value than problems (Aronsson, Perttu, 2000). Since agricultural bioenergy systems should be more profitable than those of food crops to motivate farmers to cultivate such crops, the application of residues rich in nutrients, e.g. wastewater or sewage sludge, has been identified as an attractive method for achieving environmental and energy goals, while simultaneously increasing farmers' income (Dimitriou et al., 2011). The application of wastewater sludge in agricultural lands is governed by the Council Directive 85/278 (Council of European Communities, 1986). This Directive is a legal framework document, which regulates the application of wastewater sludge in the agriculture of EC countries, but every country is allowed to introduce more strict legislative measures. In Bulgaria, the basic document regulating sludge utilisation is the "Instruction for the order and manner of utilisation of the sludge from purified wastewaters through their use in agriculture", adopted with government decree 339/14.12.2004, which has been harmonised with Directive 85/278. The utilisation of wastewater sludge for fertilisation of energy plantations in Bulgaria is still unknown. Therefore, there is a lack of crucial knowledge about the impact on woody plants growth, biomass production and the safest and most beneficial way of the wastewater sludge utilisation. In this context, pot studies could generate useful information in a short period of time and at low costs (Weih, 2001)

Bearing this in mind, the pilot study aimed at adding new insights on assessing the effect of wastewater sludge on the early growth and physiological performance of poplar and willow pot-grown plants in greenhouse conditions.

Material and Methods

Two hybrid clones (I-214, I-45/51) of black poplar (*Populus x euroamericana* (Dode) Guinier) and white willow (*S. alba* L.) from the collection of the Experimental Station for Fast-growing Tree species, Svishtov (the plant material is sourced to the nursery stock), were included in the experiment. In order to form a growing substrate, alluvial soil was mixed with stabilised dewatered sludge from the wastewater treatment plant Kubratovo. Amounts of wastewater sludge were added to the soil to obtain four treatments of 0% (control), 7.5%, 15%, 22.5% and 30% (w:w) wastewater sludge relative participation in the potting mixture. The experiment was carried out in greenhouse conditions under natural photoperiod with mean (night-day) temperatures of 18-32 °C and air humidity of 60-80%. During the growing season, plants were watered as needed and not fertilised.

For analysis of the wastewater sludge, the following methods were used:

- Determination of the pH reaction - *BS EN 12176:2000;*
- Determination of dry residue and water content - *BS EN 12880:2003;*
- Determination of total organic carbon - *BS EN 13137:2005;*
- Determination of total nitrogen - *BS ISO 11261:2002;*
- Determination of mineral nitrogen forms: ammonium / NH_4^+-N / and nitrate / NO_3 - N / N - *BS ISO 14255:2002;*
- Determination of mobile forms of phosphorus /P / - *BS ISO 11263:2002;*
- Determination of a mobile potassium / K / - *determination is carried out by methods of M. Milcheva used in ISSAPP "N.Poushkarov";*
- Determination of the solubility of sulphur /S/ as sulphate / SO_4 / *-VM-1: 2007;*
- Determination of total phosphorus content P, K, Ca, Mg, heavy metals - Cd, Cr, Ni, Cu, Zn, Pb, Hg, As - *BS EN-13346: 2000, BM-1: 2007;*
- Determination of persistent organic compounds /POPs/;
- Determination of polycyclic aromatic hydrocarbons /PAB/ - *BS ISO 17993:2003*;
- Determination of polychlorinated biphenyls /PCBs/ - *BS ISO / CDIS 10382:2002;*
- Detection and identification of microbiological indicators: Spores and sulphite reducing anaerobes - *BS EN 26461-2:2004;*
- *E. coli* and *coli*-forms - *BS EN ISO 9308-1:2004.*

The study was launched in April with standard hardwood cuttings (15-20 mm in length). It was designed as a fully randomised experiment, with every variant represented by 30 plants. Plastic pots (7.5l) were used as experimental vessels, with five cuttings planted per pot. The biometric measurements were done at the middle of the vegetation season (July). Survival frequency (%) and height (cm) of poplars; survival frequency (%, 1st year), height (cm, 1st and 2nd year) and number of resprouted shoots (2nd year) of willows were measured. The willow plants were cut at the end of the first vegetation season.

The CO_2 gas-exchange was measured with a portable infrared gas analyzer Li 6400 (LiCor Inc., Lincoln, NE, USA). The measurements (net CO_2 assimilation rate, transpiration and stomatal conductance) were performed in the middle of the vegetation season (July) between 10.00 and 13.00h. Only green and healthy leaves were selected for that purpose. The measurements were performed under the following conditions in the leaf chamber: rate of photosynthetically active radiation of 1000 $\mu mol.m^{-2}.s^{-1}$, CO_2

concentration of 350 ppm, air temperature of 25°C and relative humidity of 50 %. All the measurements were done after the values of the variation coefficient reached values < 0.5%

Analysis of variance (ANOVA) was used to assess the effect of the fixed factors (treatment, clone) and interaction between them. In case of significant factorial effects, Duncan's multiple-range test ($P < 0.05$) was used to determine the significant differences among the means. The statistical analyses were conducted with SPSS software (SPSS 19.0, 2010, Chicago, IL).

Results and Discussion

The results from the chemical analyses of the sludge are presented in Table 1. The chemical composition of the wastewater affects the dewatering properties of the sludge, the processes of its biological stabilisation and its utilisation as a soil improver. The wastewater sludge contains significant amounts of substances with both organic and mineral origin.

Sludge stabilisation treatment changes its chemical composition and often reduces its fertilising value as well. The dry matter content of the sludge was 66.96% and the pH reaction was neutral. The laboratory results showed that the sludge had substantial amounts of total nitrogen (0.79%), phosphorous (0.37%) and potassium (0.18%). The chemical forms of nitrogen are of main importance in terms of its use for agricultural purposes (Yuan, Peng, 2017). They are defined as organic and mineral. Due to biochemical processes, the organic nitrogen in the sludge partially passes into a mineral form absorbable for the plants, namely ammonium ($-NH_4$) and nitrate ($-NO_3$) salts. Actually, the latter mobile macronutrients forms, together with mobile P and K, are those utilised by the plants (Zhang et al., 2017). The amount of organic carbon in the sludge was 5.63% and its role in compensating the carbon supply in soil was important. The content of potassium (K) was very low - below 1%, typically about 0.2% of the absolutely dry matter of the sludge. The low concentrations of potassium could be related to the increased solubility of potassium salts, which remained in the water released during the sludge treatment. Approximately two-thirds (2/3) of the phosphorus in the sludge was in an organic form. The content was subject to fluctuations, thus having different availability for the plants. During the processes of drying and stabilisation of sludge on the drying fields, the values of phosphorus became smaller. The contents of calcium and magnesium were slightly higher, which might be associated with the lime treatment during processing of the wastewater sludge. Typically, the total sulphur content was between 1 ÷ 2 to 4% from the absolute dry matter of the sludge and was also affected by the process of sludge drying. The content of the total sulphur in the dehydrated sludge was low, presented partly in inorganic form as sulphate ($-SO_4$) and less frequently as sulphide (-S).

The research data for heavy metals content of the sludge showed that the reported values were below the maximum admissible concentration (MAC) as per the Regulation on the use of sludge in agriculture of 14. 12. 2004 (Turovskiy, 1988).

Table 1. Chemical and agrochemical characteristics of the wastewater sludge.

Indicators	Sludge stored over 1 year	Indicators	Sludge stored over 1 year
Dry matter (%)	66,96	Mg (%)	1.0
pH/H_20/	7.34	S-SO_4 (%)	0.7
Organic carbon (%)	5.63	Pb (mg/kg DW)	78
Total N (%)	0.79	Cd (mg/kg DW)	1
N-NH_4 (mg/kg DW)	50	Cu (mg/kg DW)	179
N-NO_3 (mg/kg DW)	291	Ni (mg/kg DW)	24
Total P (%)	0.37	Cr (mg/kg DW)	35
Total K (%)	0.18	Zn (mg/kg DW)	461
Mobile P (mg/kg DW)	210	As (mg/kg DW)	<5
Mobile K (mg/kg DW)	500	Hg (mg/kg DW)	<1
Ca (%)	4.24		

Table 2. ANOVA of the effect of sludge treatment and clone affiliation on the height growth of poplar plants.

Source	Sum of squares	df	Mean square	F	Sig.
Corrected model	4868.632	9	540.959	9.061	<0.001
Intercept	51258.372	1	51258.372	858.572	<0.001
Clone	2461.044	1	2461.044	41.222	<0.001
Treatment	1738.315.	4	434.579	7.279	<0.001
Clone x Treatment	231.280	4	57.820	0.968	0.426
Error	12000.074	201	59.702		
Total	74683.650	211			

The existence of elevated levels of heavy metals like cadmium, copper, nickel, lead, zinc, chromium, mercury and arsenic can hinder or stop the biological processes necessary for stabilisation and disinfection of sludge. These elements can also disrupt and slow down the mineralisation of organic matter and the transformation and redistribution of nutritious elements (Panayotova, Zlatareva, 2008).

Several months after the experiment was initiated the survival percentage of the poplar clones varied between 50.0 - 80.0% for clone I-45/51 and 43.3 - 90.0% for clone I-214 respectively, with the lowest values registered at 30.0% sludge dose in both the clones. The survival of the willow plants was higher, with the lowest survival frequency (80.0%) being observed again at the highest percentage of sludge application (30.0%).

The factorial interaction between the treatment dose and clonal origin had an insignificant effect on the height of the poplar plants, while both the sludge treatment and clone origin alone ($P < 0.001$) affected it significantly (Table 2).

The clone I-214 substantially outperformed in height the clone I-45/51 (19.8 and 12.7 cm, respectively). The biggest average height among the treated plants was regis-

tered at the lowest treatment dose (7.5%), which did not differ significantly from the control. The lowest height growth was recorded at the biggest sludge application dose (30.0%, Fig. 1).

The sludge treatment had no significant effect on the height of the willow plants, but considerably ($P < 0.001$) affected the number of the resprouted shoots during the second vegetation season, with the highest value being measured at the maximal treatment dose (30.0%, Table 3).

The application of wastewater sewage sludge in poor-quality soils resulted in a large increase of biomass of the willow (*S. viminalis* L.) by creating improved growth conditions associated with protection against oxidative damage, efficient functioning of the antioxidant system and maintenance of the osmotic balance between the soil environment and the plant roots (Wyrwicka, Urbaniak, 2017). However, while having a positive effect on growth and nutritional status, the application of biosolids (liquid and dry sewage sludge) on one-year old holm oak (*Q. ilex* L.) and Aleppo pine (*P. halepensis* Mill.) seedlings provoked differential responses and contrasting changes in morpho-functional traits (Valdecantos et al., 2011). In an experiment on the treatment efficiency and plant response of poplars and willows in lysimeters after application of wastewater and sewage sludge, Dimitriou, Aronsson (2011) found that irrigation with wastewater enhanced plant growth. Soudani et al. (2017) reported that plants of *Eucalyptus calmadulensis* treated with municipal wastewater sludge showed 20% better growth and 40% higher leaf numbers, while no negative effects on plants' health were observed. In a study based on the use of alvar soil and peat as growth substrates in combination with several wastewater sludge doses for treatment of 2-year old birch seedlings, Pikka (2006) concluded that the addition of wastewater sludge significantly affected the growth of

Table 3. ANOVA of the effect of treatment dose on height and number of resprouted willow shoots.

	Source	Sum of Squares	Df	Mean Square	F	Sig.
Height 1st year	Between groups	1355.293	4	338.823	1.234	0.299
	Within groups	36784.161	134	274.509		
	Total	38139.454	138			
Height 2nd year	Between groups	826.551	4	206.638	0.844	0.503
	Within groups	14938.931	61	244.901		
	Total	15675.481	65			
Shoot number	Between groups	5.940	4	1.485	5.909	<0.001
	Within groups	15.332	61	0.251		
	Total	21.271	65			

plants. The variants with mineral soil in combination with wastewater sludge provided more stressful environment for the plants than those using peat substrate. As result, birch plants growing on peat significantly exceeded those growing on alvar soil with respect to height, height increment and root collar diameter, as well as dry and fresh mass of stems and branches.

It was found that both the treatment dose and clone origin, as well as the interaction between them, had no significant effect on the photosynthetic activity of the poplar plants, with clone I-214 surpassing clone I-45/51 in values at all treatment doses (Fig. 2A). In a similar way, the stomatal conductance and transpiration were not significantly affected by both the clonal origin and wastewater sludge treatment as well as their interaction. However, the initial physiological responses at the different clones followed distinct behaviour patterns, with clone I-45-51 displaying a raising trend with increasing treatment doses and clone I-214 showing highest values at lower sludge doses (Fig. 2B-C).

The sludge treatment significantly ($P < 0.01$) increased the net CO_2 assimilation rate of the willow plants, with the value at the biggest dose (30.0%) being substantially higher as compared to the control (Fig. 3). However, the stomatal conductance and transpiration were not considerably influenced by the sludge treatment. Maximal average values were found at the highest participation of the sludge supplement, respectively 0.042 mmol $m^{-2}.s^{-1}$ and 1.171 mmol $m^{-2}.s^{-1}$.

By studying the shoot growth and photosynthetic dynamics in alpine willow (*S. glauca* L.) at increased nitrogen availability as a result of fertilisation, it was possible to conclude that the species responded primarily by increasing the leaf area per shoot rather than by increasing leaf photosynthetic capacity (Bowman, Conant, 1994). Moreover, the growth-based nitrogen use efficiency (NUE) response was not related to the nitrogen fertilisation; instead, its variation was population-specific. Our results demonstrate that the early growth height of the experimental poplar clones was significantly affected by the clone origin and wastewater sludge application. The latter did not influence the height of the willow plants either during the establishment or during the following year, but considerably increased the number of resprouted shoots during the second vegetation season.

According to Barigah et al. (1994), the controversy in results concerning correlation between the net photosynthetic rate and biomass productivity is due to difficulties in measuring the gas exchange rate of comparable leaves in different genotypes, the phenological and physiological changes during the growing season and the distribution of the photoassimilates within the tree. In our study with poplars, none of the studied physiological indices was significantly affected by the sludge application, while the particular clones responded differentially following distinct patterns of variation. Only the net photosynthetic activity of willow plants was substantially affected by the wastewater sludge supplement, particularly at higher sludge doses where plants displayed substantially higher net CO_2 assimilation rates. However, a study that explored leaf photosynthetic properties in a willow plantation found that the soil fertilisation did not enhance the leaf photosynthetic capacity (Merilo et al., 2006). Similarly, in a field study four poplar genotypes belonging to different species and with a different genetic background grown in short-rotation coppice regime showed contrasting physiological responses to environmental drivers and soil conditions (Navarro et al., 2017).

Conclusions

The treatment efficiency of wastewater sludge was dependent on the species and clone choice. However, more research is needed to clarify the role of other factors like the soil type, the chemical and agrochemical characterisation of the sludge, the time of application, etc. While increasing the treatment dose induced a positive response in the number of resprouted willow shoots, it was not efficient in relation to the stem height of both poplar and willow plants. Although the wastewater sludge application did not significantly affect stomatal conductance and transpiration, it enhanced net CO_2 assimilation rate in both species.

Use of wastewater municipal sludge as a soil fertilizer in short-rotation woody energy plantations is probably one of the most rational ways to utilise it without risk of transfer of toxic compounds into the human food chain. The assessment of efficiency of wastewater sludge application needs more extended field studies taking into consideration a broad set of additional factors. The proper choice and application could appreciably reduce the use of conventional fertilizers and the common price of biomass production.

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